TRANSMITTAL OF APPEAL BRIEF (Large Entity)

Docket No. **PB0004**

In Re Application Of: **Sharron Gaynor Penn**

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
09/774,203	01/29/2001	Lori A. Clow	22840	1631	7320

Invention: **Methods and Apparatus for Predicting, Confirming and Displaying Functional Information Derived from Genomic Sequence**

COMMISSIONER FOR PATENTS:

Transmitted herewith in triplicate is the Appeal Brief in this application, with respect to the Notice of Appeal filed on **August 22, 2005**

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Dated: **October 19, 2005**

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Melissa Leck

Typed or Printed Name of Person Mailing Correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appl. No.	:	09/774,203	Confirmation No.: 7320
Applicant	:	Sharron G. Penn, et al.	
Filed	:	January 29, 2001	
TC/A.U.	:	1631	
Examiner	:	Lori A. Clow	
Docket No.	:	PB0004	
Customer No.	:	22840	

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Alexandria, Virginia 22313-1450

October 19, 2005

APPEAL BRIEF

Sir:

Appellants submit this Appeal Brief in triplicate, appealing from the May 19, 2005, rejection of the Primary Examiner, finally rejecting claims 61-67, 70-81 and 93-104 in the captioned application. The Notice of Appeal was filed on August 22, 2005, which contained authorization to charge the "Appeal Fee" to Appellants' Deposit Account. Filed concurrently herewith is the "Transmittal of Appeal Brief (Large Entity)", in duplicate, which contains authorization to charge the fee for filing the Appeal Brief to Appellants' Deposit Account.

Real Party in Interest

Amersham plc, owners of the captioned application, is the real parties in interest to this appeal.

Related Appeals and Interferences

There are no other appeals or interferences related to the instant appeal.

Status of Claims

Claims 1-14 and 68-69 have been cancelled. Claims 15-60, and 82-92 are withdrawn from consideration. Claims 61-67, 70-81 and 93-104 are currently under examination and being finally rejected. Claims 61-67, 70-81 and 93-104 are appealed and are reproduced in the Claims Appendix, attached hereto.

Status of Amendments

Appellants did not request any amendment after Examiner's final rejection of all claims.

Summary of Claimed Subject Matter

The instant invention relates to methods and apparatus for predicting, confirming and displaying functional information derived from genomic sequence. In particular, the independent claim under appeal (claim 61) relates to a single exon nucleic acid microarray, comprising a plurality of nucleic acid probes addressably disposed on a substrate. The probes on the microarray each includes genomic sequence of at least one

predicted exon of a eukaryotic genome, with at least 50% of the probes include genomic sequence of no more than one exon of the eukaryotic genome, and the plurality of nucleic acid probes averages at least 50 polynucleotides in length. The eukaryotic genome is defined as a genome that averages at least one intron per gene.

Independent claim 61 and claims 62-67, 70-81 and 93-104 depending thereon, are directed to the single exon nucleic acid microarray discussed by Appellants at page 30, line 18, through page 38, line 24, of the specification.

Grounds of Rejection to be Reviewed on Appeal

1. Whether claims 61-67, 70-81 and 93-104 are properly rejected under 35 U.S.C. § 101 as lacking patentable utility; and accordingly, whether these claims are properly rejected under 35 U.S.C. § 112, first paragraph.

Argument

1. Claims 61-67, 70-81 and 93-104 are not properly rejected under 35 U.S.C. § 101 as lacking patentable utility; and accordingly, these claims are not properly rejected under 35 U.S.C. § 112, first paragraph.

In a final office action dated May 19, 2005, the Examiner has finally rejected claims 61-67, 70-81 and 93-104 under 35 U.S.C. § 101 as lacking patentable utility; and accompanied by a rejection of the claims under 35 U.S.C. § 112, first paragraph.

Appellants respectfully submit that the claimed microarrays have a well-established utility. Appellants assert that the claimed invention relates to research tools not unlike those research tools listed by the Office, as clearly useful, in the most recent

Manual of Patent Examining Procedure (MPEP), relevant section of which is quoted hereinbelow:

> Some confusion can result when one attempts to label certain types of inventions as not being capable of having a specific and substantial utility based on the setting in which the invention is to be used. One example is inventions to be used in a research or laboratory setting. Many research tools such as gas chromatographs, screening assays, and nucleotide sequencing techniques have a clear, specific and unquestionable utility (e.g., they are useful in analyzing compounds). An assessment that focuses on whether an invention is useful only in a research setting thus does not address whether the invention is in fact "useful" in a patent sense.

See MPEP 2107.01, page 2100-33 (Rev. 3, August 2005). Appellants submit that the claimed microarrays clearly belong to such techniques as screening assays and nucleotide sequencing, and thus have a clear, specific and unquestionable utility. Indeed, Appellants submit that a brief review of the microarray field, including the various utilities, was given in the background section of the specification (page 6, line 19 through page 7, line 22). Further, a search of the biomedical article collection (PubMed) of the National Library of Medicine identified 36 review articles related to the usefulness of microarray technology, published in the English language before the filing date of the instant application. Many of these review articles contain detailed discussion of the various utilities of microarrays.

Appellants hereby enclose, with the instant brief, one of the review articles identified during this search, entitled "Microarrays: biotechnology's discovery platform for functions genomics", by Schena, M. et al., *Trends in Biotechnology*, 16(7): 301 – 306, 1998. In the review, Schena et al. gave a review of the state of the art of the microarray industry. They state: "Advances in microarray technology enable massive parallel mining of biological data, with biological chips providing hybridization-based expression

monitoring, polymorphism detection and genotyping on a genomic scale. Microarrays containing sequences representative of all human genes may soon permit the expression analysis of the entire human genome in a single reaction. These 'genome chips' will provide unprecedented access to key areas of human health, including disease prognosis and diagnosis, drug discovery, toxicology, aging, and mental illness. Microarray technology is rapidly becoming a central platform for functional genomics." (Abstract). They also compile a list of key companies providing products and services for microarray research and development (see Table 1), including such companies as Affymetrix, Genometrix, Hewlett-Packard, Nanogen, Synteni, as well as Amersham and Molecular Dynamics. The industry has since grown substantially larger and remains one of the top growth areas in the biotechnology industry.

There are also a great number of additional publications that comment on the general utility of the microarray platforms. Appellants include one of these articles by R.W. Wallace entitled "DNA on a chip: serving up the genome for diagnostics and research" *Molecular Medicine Today*, 3(9):384-389, 1997. The Wallace reference also summarized the industrial applicability/utility of the microarray platforms (see Abstract and the entire article). Appellants respectfully submit that because cDNA and oligo-nucleotide microarrays have well-established utility, it is apparent to the artisan of an ordinary skill in the microarray field that the instantly claimed novel nucleic acid microarrays have well-established utility, as a platform for high throughput gene and exon discovery, for expression analysis of genes and alternative splicing analysis of exons, as well as for identifying gene and exon expression patterns. These are utilities

that benefit greatly to the human health, including drug discovery, toxicity, disease prognosis and diagnosis. Appellants submit that these are credible utilities.

As summarized in the "Summary of Claimed Subject Matter" section hereinabove, the nucleic acid microarrays of the instant application contain probes derived from genomic sequence of at least one predicted exon of a eukaryotic genome. The probes on the claimed microarrays are not from random fragments of genomic DNA from open reading frames of a eukaryotic genome. The probes are identified by various gene prediction programs and/or cross species comparative genomic sequence analysis. The claimed microarrays with these selected probes provide a valuable resource for high throughput gene discovery, the identification of alternatively spliced exons within a gene, the confirmation of predicted genes and exons, as well as providing expression verified single exon probes. Appellants again respectfully submit that these are "well-established" utilities. In addition, Appellants submit that specific, substantial utilities are disclosed in the instant application specification, and these utilities are credible.

Appellants also wish to reiterate again, hereinbelow, the substantial and specific utilities disclosed in the responses. One of the utilities disclosed in the instant application for the claimed microarrays is high throughput gene discovery. Appellants "have used the methods and apparatus of the present invention to identify more than 15,000 exons in human genomic sequence whose expression we have confirmed in at least one human tissue or cell type. Fully two-thirds of the exons belong to genes that were not at the time of our discovery represented in existing public expression (EST, cDNA) databases, making the methods and apparatus of the present invention extremely powerful tools for novel gene discovery" (page 24 line 33 through page 25, line 8, also page 61, lines 24 –

32). "(T)he observation that 1/3 of the arrayed genes exist in expression databases provides powerful confirmation of the power of our methodology — which combines bioinformatic prediction with expression confirmation using genome-derived single exon microarrays — to identify novel genes from raw genomic data" (page 83, line 29, through page 84, line 2). An artisan of ordinary skill in the genomics art would immediately appreciate that the two-thirds of the exons mentioned hereinabove belong to novel genes not known by the public at the time. The microarray of the instant invention is thus a useful tool for high throughput gene discovery from any eukaryotic genome averaging at least one intron per gene.

Another utility disclosed in the instant application for the claimed microarrays is the identification of alternatively spliced isoforms of genes among the large number of various cell types, developmental stages, and more importantly, physiologic conditions. The microarrays of the instant application prove to be "exceedingly useful in the high throughput identification of a large variety of alternative splice events in eukaryotic cells and tissues" (page 25 lines 9 – 17 and page 29 lines 7 – 18).

Another utility disclosed in the instant application for the claimed microarray is for verifying the expression of putative exons or genes predicted from genomic sequence (page 30, lines 18–26 and page 47, line 27 through page 48, line 9). The expression verified sequences are useful as gene-specific probes (page 28, lines 30–31), and for gene discovery. Indeed, the utilities of the instant invention in gene discovery were initially proven in the section entitled "Verification of Gene Expression" of the instant application (see page 83).

As stated hereinabove, the claimed inventions have well established utility. The specification also described multiple specific, substantial, credible utilities for the claimed invention. Appellants respectfully submit that the claimed nucleic acid microarrays satisfy the utility requirements of 35 USC 101. Appellants also submit that, as such, the claimed microarrays satisfy the 35 USC 112, first paragraph. One skilled in the art at the time of filing knew how to make and use the claimed invention.

Conclusion

In view of the foregoing, Appellants respectfully assert that the Examiner's rejection cannot be sustained and respectfully requests the reversal of the rejection.

Respectfully submitted,

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Signature: _____

Name: Melissa Leck

CLAIMS APPENDIX

The Rejected Claims

Claims 1-14 (cancelled)

Claims 15-60 (withdrawn)

Claim 61 (previously presented): A single exon nucleic acid microarray, comprising:

a plurality of nucleic acid probes addressably disposed upon a substrate,

wherein each of said probes include genomic sequence of at least one predicted exon of a eukaryotic genome, at least 50% of said probes include genomic sequence of no more than one said exon of said eukaryotic genome, said eukaryotic genome averaging at least one intron per gene, and wherein said plurality of nucleic acid probes averages at least 50 nt in length.

Claim 62 (previously presented): The microarray of claim 61, wherein at least 75% of said nucleic acid probes include genomic sequence predicted to contribute to no more than one exon of a eukaryotic genome.

Claim 63 (previously presented): The microarray of claim 61, wherein at least 90% of the probes of said microarray include genomic sequence predicted to contribute to no more than one exon of a eukaryotic genome.

Claim 64 (previously presented): The microarray of claim 61, wherein at least 95% of the probes of said microarray include genomic sequence predicted to contribute to no more than one exon of a eukaryotic genome.

Claim 65 (previously presented): The microarray of claim 61, wherein said microarray has probes that collectively include exons predicted from all chromosomes of a eukaryotic genome.

Claim 66 (previously presented): The microarray of claim 61, wherein said eukaryotic genome is a human genome.

Claim 67 (previously presented): The microarray of claim 65, wherein said eukaryotic genome is a human genome.

Claims 68-69 (cancelled)

Claim 70 (previously presented): The microarray of claim 61, wherein each of said predicted exons is represented by a plurality of probes on said array.

Claim 71 (previously presented): The microarray of claim 61, wherein said microarray includes between 5,000 and 19,000 probes.

Claim 72 (previously presented): The microarray of claim 61, wherein the genomic sequence included within said probes is further selected based upon considerations of base composition and/or hybridization binding stringency.

Claim 73 (previously presented): The microarray of claim 61, wherein said probes have been amplified from genomic DNA.

Claim 74 (previously presented): The microarray of claim 61, wherein said probes have been chemically synthesized.

Claim 75 (previously presented): The microarray of claim 61, wherein said probes are noncovalently attached to the substrate of said microarray.

Claim 76 (previously presented): The microarray of claim 61, wherein said probes are covalently attached to the substrate of said microarray.

Claim 77 (previously presented): The microarray of claim 61, wherein said probes are disposed on said microarray substrate by ink jet.

Claim 78 (previously presented): The microarray of claim 61, wherein said substrate is a glass slide.

Claim 79 (previously presented): The microarray of claim 61, wherein each of said probes is disposed on said array with its reverse complement.

Claim 80 (previously presented): The microarray of claim 61, further comprising negative control probes for hybridization.

Claim 81 (previously presented): The microarray of claim 61, wherein at least 50% of said exon-including nucleic acid probes comprise, contiguous to a first end of said predicted exon, a first intronic and/or intergenic sequence that is identically contiguous to said exon in said eukaryotic genome, and further comprise, contiguous to a second end of said predicted exon, a second intronic and/or intergenic sequence that is identically contiguous to said exon in said eukaryotic genome.

Claims 82-92 (withdrawn)

Claim 93 (previously presented): The microarray of claim 61, wherein said microarray includes at least 5,000 probes.

Claim 94 (previously presented): The microarray of claim 61, wherein said plurality of nucleic acid probes averages at least 100 bp in length.

Claim 95 (previously presented): The single exon nucleic acid microarray of claim 61, wherein at least 50% of said exon-including nucleic acid probes further comprise,

contiguous to a first end of said predicted exon, a first intronic and/or intergenic sequence that is identically contiguous to said exon in the genome.

Claim 96 (previously presented): The single exon nucleic acid microarray of claim 61, wherein at least 95% of said exon-including nucleic acid probes further comprise, contiguous to a first end of said predicted exon, a first intronic and/or intergenic sequence that is identically contiguous to said exon in the genome.

Claim 97 (previously presented): The single exon nucleic acid microarray of claim 61, wherein at least 95% of said exon-including nucleic acid probes comprise, contiguous to a first end of said predicted exon, a first intronic and/or intergenic sequence that is identically contiguous to said exon in the genome, and further comprise, contiguous to a second end of said predicted exon, a second intronic and/or intergenic sequence that is identically contiguous to said exon in the genome.

Claim 98 (previously presented): The single exon nucleic acid microarray of claim 61, wherein at least 50% of said exon-including nucleic acid probes are amplified from said eukaryotic genomic DNA.

Claim 99 (previously presented): The single exon nucleic acid microarray of claim 61, wherein at least 95% of said exon-including nucleic acid probes are amplified from said eukaryotic genomic DNA.

Claim 100 (previously presented): The single exon nucleic acid microarray of claim 61, wherein at least 50% of said exon-including nucleic acid probes lack homopolymeric stretches of A or T.

Claim 101 (previously presented): The single exon nucleic acid microarray of claim 61, wherein at least 95% of said exon-including nucleic acid probes lack homopolymeric stretches of A or T.

Claim 102 (previously presented): The microarray of claim 61, wherein said eukaryotic genome averages at least two introns per gene.

Claim 103 (previously presented): The microarray of claim 61, wherein said eukaryotic genome averages at least three introns per gene.

Claim 104 (previously presented): The microarray of claim 61, wherein said eukaryotic genome averages at least five introns per gene.

EVIDENCE APPENDIX

Appellants hereby append a copy of the following two articles, both relied upon by Appellants in the argument section hereinabove.

(1) Schena, M. et al., Microarrays: biotechnology's discovery platform for functions genomics, *Trends in Biotechnology*, 16(7): 301 – 306, 1998.

(2) R.W. Wallace, DNA on a chip: serving up the genome for diagnostics and research, *Molecular Medicine Today*, 3(9): 384 - 389, 1997.

Microarrays: biotechnology's discovery platform for functional genomics

Mark Schena, Renu A. Heller, Thomas P. Theriault, Ken Konrad, Eric Lachenmeier and Ronald W. Davis

Advances in microarray technology enable massive parallel mining of biological data, with biological chips providing hybridization-based expression monitoring, polymorphism detection and genotyping on a genomic scale. Microarrays containing sequences representative of all human genes may soon permit the expression analysis of the entire human genome in a single reaction. These 'genome chips' will provide unprecedented access to key areas of human health, including disease prognosis and diagnosis, drug discovery, toxicology, aging, and mental illness. Microarray technology is rapidly becoming a central platform for functional genomics.

Biological research can be viewed as an information science. Understanding the methodological architecture on which the discipline operates facilitates the efficient gathering of biological information (data). This hierarchy can be arranged in the form of an epistemological pyramid, with the tiers demarcated according to their degree of conceptual abstraction (Fig. 1a). In biological research, hypotheses are formulated using the most abstract components of the pyramid, such as world view and theory, and then tested using a large body of rules that govern the selection and use of methods and tools best suited for addressing a particular biological question (Fig. 1a). Hypothesis testing ultimately results in the accumulation of data, which, in turn, reshape the world view and theory, leading to subsequent rounds of hypothesis formulation and testing, and an enhanced understanding of a biological question.

One aspect of world view focuses on the notion that an ecosystem contains interactive components that can be arranged according to their biochemical complexity (Fig. 1b). The biochemical-complexity hierarchy helps to underscore the fact that biological systems are highly interactive. Complex organisms such as humans, for example, exist as a culture that is part of a much larger ecosystem; importantly, phenotype is determined by a combination of genetic and environmental factors (Fig. 1b). Understanding human behaviour, disease and other health issues thus requires more than just a knowledge of genes and genomes: one must understand the cellular, physiological, cultural and ecological context in which genomic instructions are being read.

The rate at which biological information is acquired depends, in part, on the research tools employed. Other factors being equal, better tools yield better data. Microarray technology represents a powerful new set of tools that allow researchers to link hypothesis testing and data (Fig. 1a). In the appropriate methodological context, data from chip-based experiments can provide significant quantitative information about important cellular pathways and processes. Microarrays allow the accumulation of large amounts of functional-genomic information by enabling the global ordering of molecules in a parallel fashion (Fig. 1c). Proper experimental design allows the delineation of respective internal and external contributions to the physiological state (Fig. 1c).

All microarray assays contain five discrete experimental steps – biological query, sample preparation, biochemical reaction, detection and data visualization and modelling[1]. Under appropriate experimental conditions[1], chips can provide a quantitative measure of the molecules present in biochemical extracts. Microarrays of complementary DNA (cDNA) sequences, for example, allow hybridization-based expression monitoring of the cognate genes[2–6]. In these assays, steady-state mRNA levels are deduced from the fluorescence intensity at each position on the microarray[2–6].

Advantages of chip assays

Microarray assays are rooted in early biochemical experiments on solid surfaces[7–9]. Although reminiscent of filter-based assays[10], chip assays are a fundamental departure from techniques that employ porous membranes. Chips allow true parallelism, miniaturization, multiplexing and automation, and these key features provide a set of performance specifications[2–6] that cannot be achieved with the earlier technologies.

Microarray assays allow massive parallel data acquisition and analysis. Parallelism greatly increases the speed of experimental progress and allows meaningful comparisons to be made between the genes or gene products represented in the microarray. Microarray assays may eventually allow the analysis of the entire human genome in a single reaction, and recent gene-expression experiments in yeast represent an important step towards this goal[11–13].

Miniaturization of conventional assays is a general trend in biomedical research[14]. Microscale assays reduce reagent consumption, minimize reaction volumes,

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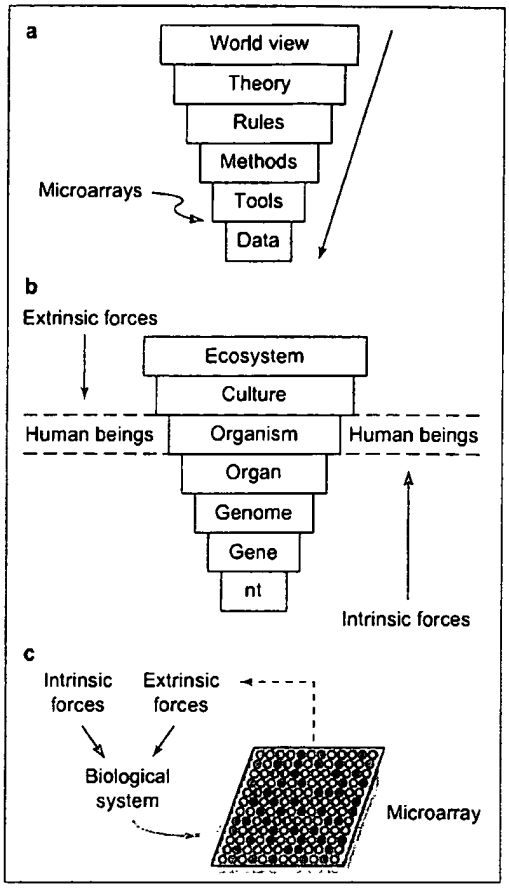

Figure 1

Methodological principles of microarray assays. (a) Methodological pyramid for microarray analysis. Levels in the pyramid are arranged in descending order of abstraction (solid arrow). Tools such as microarrays, scanners, software and biological kits occupy the second tier (open arrow). (b) Biological-information hierarchy. Levels in the pyramid are arranged in descending order of biochemical complexity. Intact organisms, such as humans, occupy the fifth tier of the pyramid. Tiers above (ecosystem and culture) and below (organ, genome, gene, nucleotide (nt)) this tier produce extrinsic (solid arrow) and intrinsic (open arrow) forces acting on organisms, respectively. (c) Microarrays in systems analysis. Intrinsic and extrinsic forces affect biological processes such as gene expression, which can be examined with microarrays.

increase the sample concentration and accelerate the reaction kinetics. Chip-based assays allow sensitive and rapid data detection with either confocal scanners[2–7,11–13] or cameras equipped with charged-coupled devices[1]. Although current microarray assays focus on nucleic acid hybridization, future studies will undoubtedly involve the parallel analysis of proteins, lipids, carbohydrates and small molecules.

Multiplexing, the process by which multiple samples are analysed in a single assay, is another enabling feature of the microarray approach. Novel labelling and detection methods, such those involving multicolour fluorescence[15], allow comparisons of multiple samples to be made on a single chip. Multiplexing increases the accuracy of comparative analysis by eliminating complicating factors such as chip-to-chip variation, discrepancies in reaction conditions and other shortcomings inherent in comparing separate experiments. The multiplexed format has already found uses in expression analysis[2–6,11–13], genotyping[16] and DNA resequencing[17].

Advanced manufacturing technologies permit the mass production of biological chips (biochips), and automation is increasing the proliferation of microarray assays by ensuring their quality, availability and affordability; as a result, biochips may eventually become commodity items akin to microchips in the computer industry. Because of the predicted central role of microarrays in biomedical research, some experts believe that the biochip revenues will eventually eclipse the sales of computer chips.

Microarray technology

Microarray-manufacturing technologies fall into two main categories – synthesis and delivery. In the synthesis approaches, microarrays are prepared in a stepwise fashion by the *in situ* synthesis of nucleic acids and other biopolymers from biochemical building blocks. With each round of synthesis, nucleotides are added to growing chains until the desired length is achieved. The delivery technologies, by contrast, use the exogenous deposition of preprepared biochemical substances for chip fabrication. Molecules such as cDNAs are amplified by PCR and purified, and small quantities are deposited onto known locations using a variety of delivery technologies.

The key technical parameters for evaluating both the synthesis and delivery technologies include microarray density and design, biochemical composition and versatility, reproducibility, throughput, quality, cost and ease of prototyping. Three types of advanced technologies have emerged as early favourites in automated microarray production (Fig. 2).

Photolithography

One novel synthesis technology, developed by Fodor and colleagues (Affymetrix, Santa Clara, CA, USA) combines photolithography technology from the semiconductor industry with DNA-synthetic chemistry[4,7,13,14,17] to enable high-density oligonucleotide-microarray manufacture (Fig. 2a). A key advantage of this approach over nonsynthetic methods is that photoprotected versions of the four DNA building blocks allow chips to be manufactured directly from sequence databases[4,7,13,14,17], thereby removing the uncertain and burdensome aspects of sample handling and tracking. Another advantage of the photolithographic approach is that the use of synthetic reagents minimizes chip-to-chip variation by ensuring a high degree of precision in each coupling cycle. One disadvantage of this approach is, however, the need for photomasks, which are expensive and time-consuming to design and build.

Affymetrix chips currently contain as many as 400 000 groups of oligonucleotides or features in an area of ~1.6 cm^2, with each feature containing approximately ten million oligonucleotides of a given sequence. Oligonucleotides are anchored at the 3′ end, thereby maximizing the availability of single-stranded

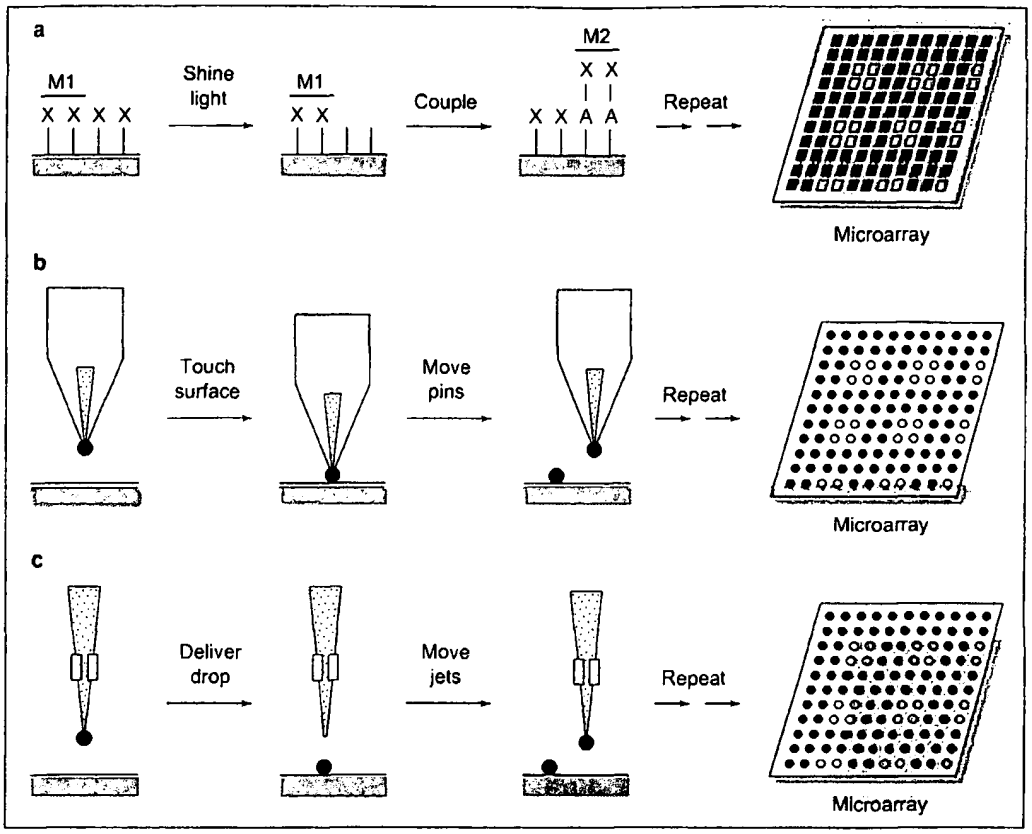

Figure 2

Microarray technologies; three approaches to microarray manufacturing are depicted. (a) Photolithography: a glass wafer modified with photolabile protecting groups (X) is selectively activated for DNA synthesis by shining light through a photomask (M1). The wafer is then flooded with a photoprotected DNA base (A–X), resulting in spatially defined coupling on the chip surface. A second photomask (M2) is used to deprotect defined regions of the wafer. Repeated deprotection and coupling cycles enable the preparation of high-density oligonucleotide microarrays. (b) Mechanical microspotting: a biochemical sample is loaded into a spotting pin by capillary action, and a small volume is transferred to a solid surface by physical contact between the pin and the solid substrate. After the first spotting cycle, the pin is washed and a second sample is loaded and deposited to an adjacent address. Robotic control systems and multiplexed printheads allow automated microarray fabrication. (c) Ink jetting: a biochemical sample is loaded into a miniature nozzle equipped with a piezoelectric fitting (rectangles) and an electrical current is used to expel a precise amount of liquid from the jet onto the substrate. After the first jetting step, the jet is washed and a second sample is loaded and deposited to an adjacent address. A repeated series of cycles with multiple jets enables rapid microarray production.

nucleic acid for hybridization. Future modifications of the Affymetrix approach, such as the use of acid-resist technology, may allow microarray manufacturing without the need for photomasks (J. Beecher, pers. commun.). Steady improvements in coupling efficiency, density and biochemical diversity will ensure the viability of the Affymetrix platform in a competitive micorarray marketplace.

Mechanical microspotting

A second robust set of technologies are the mechanical microspotting approaches, an original version of which was developed by Shalon and Brown[2,16] and later commercialized at Synteni (Fremont, CA, USA). Microspotting, a miniaturized version of earlier DNA-spotting techniques[10], encompasses a family of related deposition technologies that enable automated microarray production by printing small quantities of premade biochemical substances onto solid surfaces (Fig. 2b). Printing is accomplished by direct surface contact between the printing substrate and a delivery mechanism that contains an array of tweezers, pins or capillaries that serve to transfer the biochemical samples to the surface (Fig. 2b).

Some of the advantages of the microspotting technologies include ease of prototyping and therefore rapid implementation, low cost and versatility. One disadvantage of microspotting is that each sample must be synthesized, purified and stored prior to microarray fabrication. The microspotted microarrays currently manufactured at Synteni contain as many as 10 000 groups of cDNAs in an area of ~3.6 cm^2; each cDNA feature permits the expression monitoring of its cognate human gene. A set of four Synteni microarrays should thus allow the expression monitoring of ~40 000 human genes, the number of unique expressed

Table 1. Microarray Industry

Company	Contact information	Key products and services
Affymetrix	Santa Clara, CA, USA	GeneChip™ technology, microarray contract services, complete microarray systems
Alphagene	Woburn, MA, USA	AlphaGenomics™, full-length cDNAs, microarray contract services
Amersham	Amersham, UK	CyDye™ fluorescent-labelling reagents
Biodot	Irvine, CA, USA	Ink-jetting technology, microarray instrumentation
CLONTECH Labs	Palo Alto, CA, USA	Technology Access Program, gene-expression reagents
General Scanning	Watertown, MA, USA	Confocal-scanning instrumentation
Genetix	Dorset, UK	Microspotting instrumentation
Genome Systems	St Louis, MO, USA	Expressed-sequence-tag (EST) libraries
Genometrix	The Woodlands, TX, USA	Microarray technology platform, contract services
Genomic Instrumentation Services	Menlo Park, CA, USA	Development Partners Program, microarray instrumentation
Hewlett-Packard	Palo Alto, CA, USA	GeneArray™, confocal-scanning instruments (Affymetrix)
Hyseq	Sunnyvale, CA, USA	HyChip™ products, genomics platform, contract services
Incyte Pharmaceuticals	Palo Alto, CA, USA	LifeSeq™ database, GeneJet™ and GEM™ technology, microarray contract services
Intelligent Automation Systems	Cambridge, MA, USA	Custom automation, microarray instrumentation, contract work
Life Technologies	Gaithersburg, MD, USA	Fluorescent-labelling reagents
Molecular Applications Group	Palo Alto, CA, USA	GeneMine Pro™, data-analysis and -visualization software
Molecular Dynamics	Sunnyvale, CA, USA	Microarray Technology Access Program, complete microarray systems
Nanogen	San Diego, CA, USA	APEX™, electronic microarray technology, contract services
Norgren Systems	Palo Alto, CA, USA	CCD-based imaging, microspotting instrumentation
OncorMed	Gaithersburg, MD, USA	Cancer prognostics and diagnostics
Pangea Systems	Oakland, CA, USA	GeneWorld™, data-mining, -analysis and -management software
Protogene Laboratories	Palo Alto, CA, USA	Ink-jetting technology, microarray contract services
Qiagen	Hilden, Germany	DNA- and RNA-purification systems
Research Genetics	Huntsville, AL, USA	GenePairs™, primers and purified PCR products
Silicon Graphics	Mountain View, CA, USA	Computational hardware and software, data-visualization and -mining tools
Synteni	Fremont, CA, USA	GEM™ technology, microarray contract services
TeleChem International	San Jose, CA, USA	ArrayIt™, PCR purification systems, microspotting technology, scanners

sequences currently in public databases. Although microspotting is unlikely ever to produce the densities of photolithography, improvements in mechanical-spotting technologies will eventually allow the automated production of chips containing 100 000 features in an area of ~6.5 cm^2. Because of the ease of use and affordability, microspotting may become the microarray technology of choice for the basic research laboratory.

Ink jets

A third group of microarray technologies, the 'drop-on-demand' delivery approaches, provide another way to manufacture microarrays (Fig. 2c). The most advanced of these approaches are adaptations of the ink-jetting technologies[18–21], which utilize piezoeletric and other forms of propulsion to transfer biochemical substances from miniature nozzles to solid surfaces (Fig. 2c). Similar to the microspotting approaches, drop-on-demand technologies allow high-density gridding of virtually any biomolecule of interest, including cDNAs, genomic DNAs, antibodies and small molecules. Ink-jetting technology is being developed at several centres including Incyte Pharmaceuticals (Palo Alto, CA, USA) and Protogene (Palo Alto, CA, USA).

Although ink jetting is not currently as robust as photolithography or microspotting, this approach has been used to prepare microarrays of single cDNAs at a density of 10 000 spots cm^{-2}. Because ink jetting does not require direct surface contact, piezoelectric delivery is theoretically amenable to very high throughput. Improvements in sample loading and sample changing should, coupled with the inherent high-density capabilities of this approach, eventually enable the manufacture of complex microarrays. Piezoelectric-based delivery of phosphoramidite reagents has recently been used for the manufacture of high-density oligonucleotide microarrays[21]. The successful application of ink jetting in a gene-expression setting (Fig. 3) demonstrates the immediate utility of this technology for genome analysis.

Combinations

In view of the growing interest in microarray technology and its potential impact on drug development and disease profiling, it is unrealistic to suggest that any single enabling technology will dominate this large and diverse industry. A more balanced view suggests that each of the technologies described above, and perhaps others[22,23], will be utilized for the purpose they perform best at and will assist collectively in the proliferation of microarray assays. The burgeoning microarray industry, complete with scientific, business, financial and legal components, will soon provide a complete repertoire of technologies and services for the scientific community (Table 1).

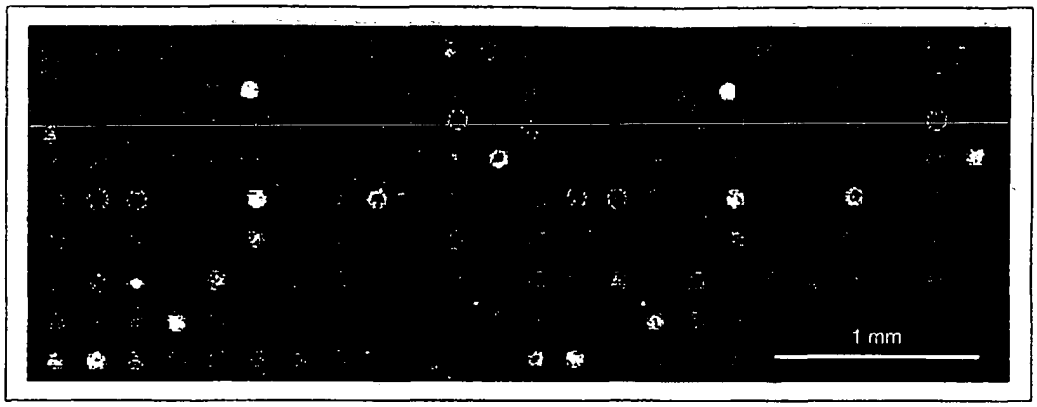

Figure 3

Gene-expression monitoring with an ink-jetted microarray. This is a fluorescent scan of a high-density microarray printed using a GeneJet™ (Incyte). Piezojet delivery of 200-pl droplets provides a density of 2500 cDNA groups cm^{-2}. Coupling of the cDNAs to the chip surface occurs via a succinimidyl-ester-displacement reaction. Array elements, printed as adjacent 9 × 12 subgrids, correspond to human cDNAs selected from the LifeSeq™ database (http://www.incyte.com/), which contains approximately three million annotated expressed sequence tags. The fluorescent sample was prepared from cultured human THP-1 cells by biotin incorporation into antisense RNA, followed by secondary labelling with Cy-5 conjugated streptavidin (Molecular Probes, Eugene, OR, USA). Fluorescent intensities, represented in a pseudocolour scale, reflect gene-expression levels.

Applications

In a similar way to the development of recombinant DNA and the polymerase chain reaction (PCR), microarrays have a large number of applications that will expand and diversify over time[24–26]. At present, expression monitoring appears to be one of the most biologically informative applications of this new technology[1–6,11–13]. For a number of reasons, chips are well suited to gene-expression analysis[1,27]; one theoretical advantage of the use of micrarrays for expression assays over some other applications is that these assays focus on the functional (expressed) segments of the genome. This aspect is important in complex systems, such as the human genome, in which the ratio of coding to noncoding DNA is low[28]. Because expressed sequences account for only ~3% of the genome, hybridization-based transcript analysis effectively reduces the complexity of the human genome by ~30-fold.

Microarrays of cDNAs provide expression information for each gene represented on the chip. A microarray of 100 000 unique human cDNAs should therefore allow the expression monitoring of the entire human genome in a single hybridization. The enormous information content of expression assays can be seen clearly when compared with chips designed for other applications, such DNA resequencing: resequencing microarrays containing 100 000 groups of oligonucleotides would allow sequence checking for 25 000 bases, or approximately 0.0008% of the human genome.

Another advantage of the expression applications is that the data serve as a direct link to function. Steady-state transcript levels provide a sensitive, global readout of the physiological state of a cells and tissue samples. This is evident in the fact that specific patterns of gene expression have been observed as function of tissue type[2], heat shock and phorbol-ester treatment[3], and a spectrum of metabolic and disease states[5,6,11–13]; moreover, these patterns are biologically informative and provide direct clues to gene function.

By correlating changes in gene expression with specific changes in physiology, it is possible to gain mechanistic insight into a broad range of biological processes (Fig. 4). Variations in gene expression in the normal population may, coupled with clinical data, have prognostic value by allowing correlations to be made between the presence of specific expression markers and disease susceptibility (Fig. 4). Altered expression patterns are expected to accompany many or all human diseases; thus, microarray analysis of diseased tissues is expected to provide a wealth of diagnostic and pathology information and yield potential drug targets as well (Fig. 4). A gene whose expression level is dramatically altered in a specific disease may provide a specific target for small-molecule inhibition. The treatment of cells with small molecules has been shown

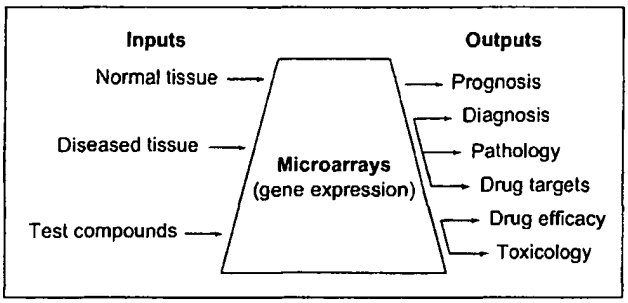

Figure 4

Microarrays for gene-expression analysis provide an integrated platform for functional genomics. Changes in the physiological state of the cells and tissues used for microarray analysis lead to specific changes in gene-expression patterns. Messenger RNA from samples of interest (inputs) is isolated, labelled and analysed by hybridization-based microarray analysis, yielding quantitative expression information for thousands of cellular genes. Expression data, coupled with other types of information (such as that obtained in clinical and pharmacological studies) can have an impact on a spectrum of research areas (outputs).

to elicit specific changes in gene expression by itself[3,4,6]; thus, because of the costly nature of clinical trials, microarray assays could provide a relatively inexpensive platform for drug screening and toxicology studies (Fig. 4).

Conclusions

The genome age will change biology forever, providing sequence blueprints for numerous bacteria, fungi, plants and animals. Whole genome sequences, the holy grail of stuctural genomics, will pave the way for functional genomics by providing the information required for microarray manufacture. Genome chips will be a focal point of functional genomics by allowing the massive parallel analysis of expression patterns, mutations and many other types of genomic information[29–33]. The data flood generated by chip-based assays will be managed by powerful bioinformatics tools capable of relational data analysis. Computer queries of gene-chip databases should eventually enable virtual biomedical research.

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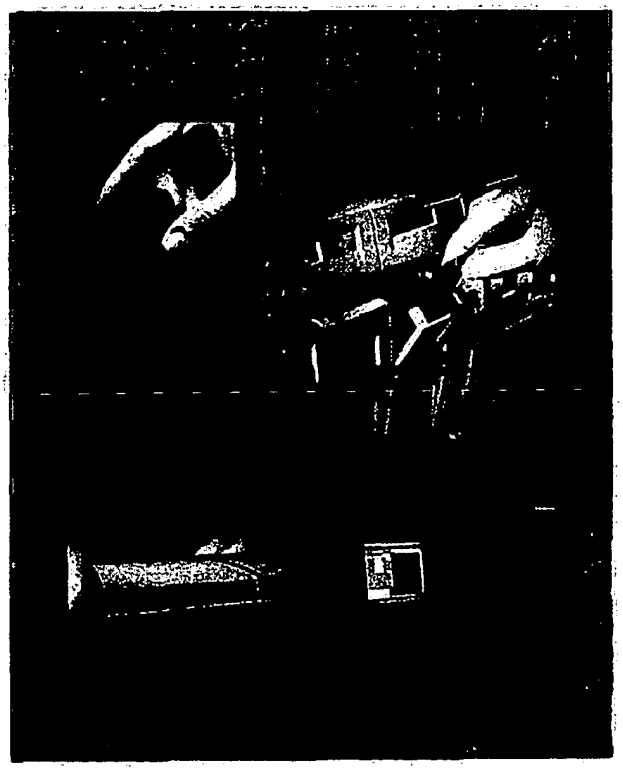
DNA on a chip: serving up the genome for diagnostics and research

Robert W. Wallace

It is expected that by the year 2003 the entire human genome will be sequenced, providing us with new insight into human disease. The amount of human sequence information that is already available (only a few percent of the total) is, however, already much greater than can be routinely evaluated with existing, commonly used diagnostic technology. New technology based on attaching DNA to a chip for parallel hybridization analysis is generating much interest in both the basic research and the clinical diagnostic community. It will increase, by orders of magnitude, our ability to evaluate an individual's genetic heritage and to conduct basic genetic research. The new technology will be a major advance in screening for genetic diseases, but the ability to treat these newly understood genetic defects will lag significantly behind the new-found diagnostic capabilities.



Images kindly provided by Affymetrix Inc., Santa Clara, CA, USA.

EXISTING diagnostic tests used to probe genetic information include the polymerase chain reaction (PCR) for amplifying specific regions of the genome, automated sequencing chemistries, automated reading of sequencing gels, and assorted variations on the classic Southern, northern or dot blot techniques to identify genes or evaluate tissue- or cell-specific gene expression. Although powerful, these assays are cumbersome, relatively low-throughput, expensive and often rely upon electrophoresis skills found mainly in specialized research or diagnostic laboratories.

The major limiting feature of these current genetic tests is that they are sequential in nature, requiring significant amounts of time and labor to evaluate just a single gene. For example, the diagnostic laboratory OncorMed (Gaithersburg, MD, USA) currently offers an evaluation of the *BRCA1* and *BRCA2* genes for mutations linked to increased incidence of breast and ovarian cancer. A complete, end-to-end evaluation of exons of these two genes currently takes up to eight weeks and costs approximately US $2100. Leslie Alexandre, OncorMed's Vice President of Corporate Affairs, predicts: 'The new DNA chip technologies, which will probe the entire sequence of *BRCA1* and *BRCA2* simultaneously, may reduce the cost by an order of magnitude or more and the turn-around time for the assay to several days.' Similar progress can be expected in the diagnosis of a wide range of other genetic conditions including: cystic fibrosis, drug resistance to HIV in AIDS, and enzyme-based drug metabolism by the liver (see below).

Not only will the new technology be a boon for diagnosis, it will also greatly speed up basic research. Mark Johnston, a yeast geneticist at Washington University School of Medicine (St Louis, MO, USA), believes that it will revolutionize the field. Linda Lutfiyya, a graduate student in Johnston's laboratory, is conducting DNA chip

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experiments in collaboration with Pat Brown's laboratory at Stanford University (Palo Alto, CA, USA) to identify yeast genes regulated by a newly identified DNA-binding protein. Brown has the 'still rare' DNA chip technology in his laboratory. According to Johnston, 'with standard techniques this would be a 2–3 year project or possibly an entire thesis project for a graduate student. With the chip technology, however, we were able to identify in a single experiment all the genes in yeast regulated by the protein.' The only problem, says Johnston, is that the technology is not readily available to everyone in the field. 'Many of my colleagues are asking how they can get access to this technology – but the line is getting pretty long.' Johnston believes that 'the technology will be exportable in a year or two' and he says he is trying to put pressure on its manufacturers because it is in great demand.

Stephen Fodor and Affymetrix

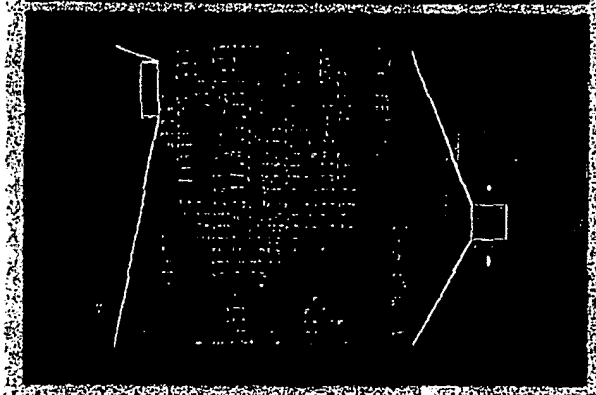
One company that has led the way with new technologies for genetic analysis is Affymetrix Inc. (Santa Clara, CA, USA), the developers of the now-famous GeneChip® array (Box 1). The technology behind the GeneChip array was invented in 1988 and developed at Affymax (Palo Alto, CA, USA), one of the early pioneers of the technologies of combinatorial chemistry (today it is a subsidiary of Glaxo Wellcome), by a team including Dr Stephen Fodor, now President and CEO of Affymetrix. Initally established as a division of Affymax, Affymetrix was established in 1991 as an entirely separate company with the GeneChip technology as its major focus (34% of Affymetrix is now owned by Glaxo Wellcome). 'Fodor is clearly the visionary behind the development of the GeneChip array,' says Thane Kreiner, Director of Marketing and Project Management for Affymetrix. While still an Affymax scientist, Fodor pursued the idea that combinatorial libraries could be built on a single chip. He originally developed chip-based peptide libraries, but 'Fodor foresaw the utility of DNA libraries in this format for looking at genetic information,' remarks Kreiner.

To produce the GeneChip array, several new technical developments were required. First, new light-activated chemistry had to be developed that could be applied to oligonucleotide synthesis, and second, a method had to be devised by which the light-activated chemistry could be applied selectively to different spatial regions of the chip. Fodor's group met the first requirement by using photolabile protective groups on the reactive end of oligonucleotides, which are removed upon exposure to light. The second requirement was met by using lithography techniques that were routinely used in the semiconductor industry to generate masks that cover and control the light exposure at different spatial regions on the chip (Refs 1–3). In their platform technology, a precise geometric pattern of hundreds of thousands of short pieces of DNA with defined sequences is built up on a small glass chip. One chip, according to Affymetrix, has the potential to analyze (in parallel) hundreds or thousands of different sequence loci on a gene.

So, are the proclamations regarding the utility of the GeneChip technology mainly hype or is it truly a revolutionary advance? 'There is some hype in the field,' says Gary Cutting, an expert in the molecular genetics of cystic fibrosis (Associate Professor of Pediatrics and Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA), 'but this is understandable; the concept parallels the computer chip. Think of how far computers have come in the last ten years. Molecular genetics has had the same pace of achievments.' He adds, 'the possibility of putting a massive number of interrogations on a single chip is very exciting and will provide us with tools that we just didn't imagine ten years ago'.

Joseph Hacia, a researcher at the National Center for Human Genome Research (Bethesda, MD, USA) has used the GeneChip technology to investigate the *BRCA1* and *BRCA2* genes[4]. He also notes that the industry's public relations departments have done a very good job

Box 1. How the Affymetrix GeneChip technology works



The GeneChip arrays are small pieces of glass wafer, approximately 1–2cm², covered with a layer of short oligonucleotide probes, arranged with precisely known nucleotide sequences. The chip is encased in a black plastic cartridge in which the reactions and washings inherent to the assay methodology take place.

At the heart of the GeneChip technology is the ability of nucleic acids to hybridize with other nucleic acids with a complementary nucleotide sequence. For example, a piece of DNA with the sequence ATGGGCT will readily bind to a stretch of DNA with the sequence TACCCGA. However, if even one nucleotide is not complementary, the binding affinity of the interaction will be greatly reduced. The strength and specificity of the interaction can be increased or decreased by altering the length of the oligonucleotides or the conditions of the hybridization reaction.

To manufacture the chip, a nucleotide containing a photolabile protective group on one of its chemically reactive ends is first coupled to the glass surface of the chip. Then a mask is placed over the chip. When the chip is illuminated with a mask in place, those regions where the mask allows light to pass to the surface of the chip have their photolabile protective groups removed. Those areas that are in the shadow of the mask retain their protective groups. After illumination, the chip is incubated with a solution of a second nucleotide, which reacts and is added to the existing oligonucleotide only in those regions where the mask allowed the light to penetrate and destroy the photolabile protective groups. Using this strategy repeatedly, each time reacting the chip with a different nucleotide and allowing different selected spatial regions of the chip to react, it is possible to produce an incredibly complex array of distinct oligonucleotides. For example, the entire set of all possible 10-nucleotide oligonucleotides (4^{10} different oligonucleotides) can be generated in only 40 photomask and nucleotide coupling cycles, providing the chip can be divided into that many distinct domains by a lithographic mask.

Table 1. DNA chip products on the market or in development

Chip name	Gene target	Application and status	Industrial partners	Ref.
GeneChip HIV PRT Assay	HIV *pol* and *pr* genes	Identification of particular strains of HIV with mutated reverse transcriptase and protease enzymes for selection of drug regimen; now on the market	Glaxo Wellcome plc (Stevenage, UK) and Affymetrix Inc. (Santa Clara, CA, USA)	8
p53 array	*TP53* gene (encodes the p53 tumor suppressor protein) and genes involved in breast and ovarian (*BRCA1* and *BRCA2*), colon, and other cancers	Cancer detection and monitoring	OncorMed, Inc. (Gaithersburg, MD, USA) and Affymetrix Inc.	4
Flu array	Selected *Haemophilus influenzae* and *Streptococcus pneumoniae* genes	Monitor expression of selected bacterial genes	Hoffman–La Roche (Basel, Switzerland) and Affymetrix Inc.	
Expression array	Human gene expression (genes from the Merck Gene Index project)	Drug discovery, diagnosis of human genetic diseases	Merck & Co, Inc. (Whitehouse Station, NJ, USA) and Affymetrix Inc.	
LifeChip arrays	Up to 1500 genes on one chip selected from Incyte's LifeSeq™ database)	Detection of various genes involved in human diseases	Incyte Pharmaceuticals, Inc. (Palo Alto, CA, USA) and Affymetrix Inc.	
Bacterial arrays	Bacterial gene expression	Diagnostic kits for bacterial identification and antibiotic resistance	bioMerieux Vitek Inc. (St Louis, MO, USA) and Affymetrix Inc.	
Cystic fibrosis array	Cystic fibrosis transmembrane conductance regulator *CFTR* gene	Diagnosis (and treatment) of cystic fibrosis	Roche Molecular Systems (Palo Alto, CA, USA) and Affymetrix Inc.	
P450 array	Polymorphic forms of the gene for the human liver enzyme cytochrome P450	Prediction of rates of metabolism of various drugs including β-blockers for cardiovascular disease, anticonvulsant medications and antidepressants	Hewlett–Packard Co. (Palo Alto, CA, USA) and Affymetrix Inc.	
Human gene array	Over 15 000 different human genes	Human gene expression for drug discovery	Genetics Institute Inc. (Cambridge, MA, USA) and Affymetrix Inc.	5
Standard GEMs	10 000 different human genes	Human gene expression for basic research and drug discovery; now on the market	Synteni and Stanford University (both Palo Alto, CA, USA)	
DNA micro-chips	Genes from bacteria, viruses and fungi	Diagnosis of infectious disease	Nanogen Inc. (San Diego, CA, USA) and Becton Dickinson Co. (Franklin Lakes, NJ, USA)	7
HP G2500A GeneArray Scanner		Instrumentation to read gene chips; now on the market	Hewlett–Packard Co. and Affymetrix Inc.	
GeneJet™	100 distinct cDNAs spotted 16 times each on a 1600 spot cm^{-2} grid	Custom low-density DNA microarrays for pharmaceutical customers; available in July 1997	Incyte Pharmaceuticals (Palo Alto, CA, USA)	

to promote the technology. But despite the resulting hype, Hacia believes the chip technology has 'the potential to have a very specific niche for scanning large pieces of DNA for alterations between individuals and to look at expression levels of different genes. It's good science; I'm not sure it's going to revolutionize life, but it is going to have an impact.'

Another application of this technology is AIDS research. The GeneChip HIV PRT Assay can rapidly identify specific mutant strains of HIV by screening the viral *pol* and *pr* genes, which encode the HIV reverse transcriptase and protease enzymes. This research tool is expected to be used to explore mutations that, one day, might provide physicians with a rationale for prescribing optimal drug therapies based on knowledge of the particular mutant strain of the virus infecting the patient.

The HIV GeneChip Assay sells for approximately US $90.00 and is designed to be used once and discarded. The instrumentation and software needed to conduct the assay and evaluate the results – the GeneChip Analysis System – is also available from Affymetrix and sells for approximately US $150 000 in the USA. According to Kreiner, reference laboratories are already offering the assay on a commercial basis for approximately US $450 per patient in 7 days. Affymetrix and other companies have a number of other products in the pipeline (see Table 1).

Currently, approximately 400 000 different oligonucleotides are arranged on a singe chip of 1 cm^2 at a resolution of about 20 μm. This resolution is well within the range that can be achieved with the current lithographic mask technology. In fact, with the most sophisticated techniques, such as electron beam lithography, it is possible to produce masks that have a resolution of approximately 250 Å to give a pattern of 10^{10} distinct domains per cm^2, which is more than enough to generate all possible combinations of 10-nucleotide oligonucleotides (probably the minimum length for a useful oligonucleotide probe). Fodor believes that this is well within the realm of possibility and that, in the future, GeneChip arrays might be produced containing up to 1.6 million distinct oligonucleotides. According to Cutting, a chip this size could be used to look for mutations in 10–20 different genes simultaneously, or to check the sequence of the entire *BRCA1* gene, which might bring the cost down to a couple of hundred dollars.

Using the DNA chip technology

Cystic fibrosis

Cystic fibrosis is the most common genetic disease in the Caucasian population. It is a recessive trait caused by mutations in both alleles of the *CFTR* gene, and approximately one in 20 Caucasians carries a disease-causing mutation in one copy of *CFTR*. The actual occurrence of cystic fibrosis is about one per 2000–2500 Caucasian births. When the *CFTR* gene was discovered, it was hoped that a highly reliable genetic test would be forthcoming. Unfortunately, the gene is large (consisting of more than 250 kilobases) and complex (containing 27 exons). The mutation responsible for about 70% of cases of cystic fibrosis results in a deletion of a phenylalanine residue at position 508 of the CFTR protein (ΔF508); the other 30% of cystic fibrosis cases result from a complex family of over 600 different mutations that are found throughout the *CFTR* gene. However, approximately 80–85% of the cases (those that are most common) can be detected by screening for just 16 mutations.

Now that these different mutations have been characterized, it has become clear that the severity of the disease correlates with the position and nature of the mutation on the *CFTR* gene. In addition, 'recent data suggest that the different therapeutic approaches for treating cystic fibrosis are more effective when matched to the appropriate genotype,' says Gary Cutting. For example, some therapies might be more effective for a protein that does not fold properly, while other therapeutic approaches might work better for proteins that fold properly but do not respond to second messengers.

Using GeneChip technology, it might be possible to test for all 600+ known mutations of the *CFTR* gene with one chip, or even to check the sequence of an individual's entire *CFTR* gene to determine whether any mutations (known or unknown) are present. Scientists at Affymetrix have already made progress in this direction. They designed a chip with 428 oligonucleotide probes to scan for many of the known point mutations in the *CFTR* gene, while another chip, with 1480 probes, can test for a wider array of mutations including deletions, insertions and base substitutions. In a study of samples from ten patients, the diagnostic results with the chips were identical to those obtained by PCR–restriction-enzyme analysis[5]. 'The chip provides the ability to not only screen for the common mutations in Caucasians, but it also allows one to look for the mutations that are common in other populations as well as those associated with milder forms of the disease,' notes Cutting. He thinks it provides more than just 'a better mouse trap'; it provides more value.

Cancer, BRCA1 and DNA chips

With the mini-explosion in the identification of genes associated with hereditary and 'spontaneous' forms of cancer, it is becoming feasible to use genetic testing to screen for the likelihood of developing some cancers or for detecting specific cancer-associated mutations in affected tissues. So far, these genes have shown a highly heterogeneous mix of mutations, which makes genetic analysis by traditional methods difficult, expensive and sometimes inconclusive. A novel, two-color fluorescence analysis using the Affymetrix GeneChip system has been used to rapidly screen the entire *BRCA1* gene for mutations[5]. This is one of the genes strongly associated with familial breast and ovarian cancer. The chip was made with an array of over 96 000 20-nucleotide oligonucleotides that were selected to screen for a wide array of the known mutations in the *BRCA1* gene. The novelty of the technique is in the use of two different populations of target DNA, each labeled with a different colored fluorescent probe. Using this approach, 14 of 15 samples from patients with known mutations were accurately diagnosed, and no false positives were found in another 20 samples that did not contain mutations.

Other companies using similar technologies

Affymetrix and HySeq Inc. (Sunnyvale, CA, USA) are currently involved in a legal battle over the patent rights to certain hybridization technologies. HySeq has a patent that, it claims, covers the use of hybridization in gene sequencing, among other things. It claims that Affymetrix is using the DNA chip technology to sequence genes and is therefore violating HySeq's patent rights. Affymetrix insists that determining the presence or absence of a particular set of mutations in a previously sequenced gene is inherently different in both principle and practice from sequencing a gene for the first time, and that its use of the technology is outside the scope of the HySeq patent. The dispute is expected to be heard in the Northern District Court of California for the first time in 1999.

A third claim to the technology has been made by Ed Southern of Oxford University (Oxford, UK), the inventor of the now classic Southern blotting technique, which was never actually patented prior to gaining wide use. Southern's claim is that the DNA chip technology is essentially the application of oligonucleotide arrays as a testing platform; Southern has been allowed a European patent for this application and has applied for a US patent.

An alternative form of DNA chip technology has been developed by Combion Inc. (Pasadena, CA, USA) in which 'ink-jet printing'

Box 2. Checking sequences on a chip

If the objective is to check the sequence of a particular gene, then it is necessary to produce a DNA chip containing families of oligonucleotides with overlapping sequences of the gene of interest. Consider a target gene that contains the sequence AATGCACTTGGA as its first 12 nucleotides. A DNA chip for checking the sequence of the target gene might use the following oligonucleotide array for hybridization:

```
TTACGTGAAC
TTACCTGAAC
TTACTTGAAC
TTACATGAAC

 TACGGGAACC
 TACGCGAACC
 TACGTGAACC
 TACGAGAACC

  ACGTGAACCT
  ACGTCAACCT
  ACGTTAACCT
  ACGTAAACCT
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Simply by determining which of the four DNA chip oligonucleotides undergoes hybridization with the fluorescently labeled target gene will reveal mutations in the sequence of the target gene at the fifth position. To examine at the sixth position, a different set of 10-nucleotide oligonucleotides must be available based upon the known sequence of the gene beginning with the number 2 position and ending at the number 11 position. This scenario is repeated to determine the nucleotide at the seventh position. By continuing down the gene in a similar manner, it is possible to quickly compare the gene sequence of an individual's gene to the known wild-type gene sequence, thereby detecting any mutations or polymorphisms that may reside in that individual's target gene. To screen every nucleotide in a gene composed of 25 000 nucleotides, a DNA chip with 100 000 different oligonucleotides would be required, well within the limits of the present technology.

techniques are used to produce low-density grids of DNA on small chips. This technology is used for both combinatorial synthesis and the placement of microarrays of DNA on the chip surface. In August 1996, Combion was purchased by Incyte Pharmaceuticals (Palo Alto, CA, USA); they have combined Combion's ability to manufacture DNA chips with Incyte's LifeSeq GeneAlbum™, a library of approximately 100 000 cDNA probes for expressed human genes. By combining the two technologies, a product termed GeneJet™ has been brought to market. The current GeneJet chip is provided by Incyte on a custom basis to pharmaceutical clients conducting gene expression research.

Synteni (Palo Alto, CA, USA) is another small biotechnology company developing the DNA chip technology, which it terms GEM. Using liquid handling techniques, Synteni places microarrays of cDNA molecules on a glass surface where they are chemically bonded. Synteni claims to have the capability to place up to 10 000 distinct cDNA elements on the surface with an element spacing of 50–200 μm. Synteni focuses on producing chips to monitor gene expression quantitatively. Currently it has a chip available that is capable of measuring the expression of 10 000 distinct human genes; its goal is eventually to market chips that will be capable of monitoring the entire human genome. According to Dari Shalon, President and founder of Synteni, with the GEM chips 'information can be obtained concerning the difference between similar cells in different metabolic states or at different points in differentiation, diseased and normal cells, and cells treated or untreated with drugs or toxic substances.'

Nanogen (San Diego, CA, USA) was formed in 1993 as an electronic bioassay company. It also uses DNA chips for the analysis of genomes, but instead of using a huge array of DNA probes (as the Affymetrix chips do), it focuses on the use of electric fields to rapidly transport, denature, hybridize and dehybridize DNA at individually addressable sites on small electronic chips. It claims that all of this can be done in 15 seconds or less on its chips, which can be used to identify mutations and polymorphisms. Nanogen has also developed integrated instrumentation in which the reactions take place and the results are read using fluorescence indicators. The company plans to focus largely on the diagnosis of infectious disease. In May 1997, they entered into a broad-based worldwide collaborative agreement with Becton Dickinson (Franklin Lakes, NJ, USA) for this purpose. This agreement will couple Nanogen's DNA micro-chip technology and instrumentation with Becton Dickinson's DNA amplification system[7].

Questions arising for molecular medicine

- Using the current fluorescent detection techniques, what will be the maximum number of oligonucleotides that can be loaded onto a single DNA chip and be resolved after hybridization? Will new detection techniques be developed that can increase this number significantly?
- How many human genes actually exist, how many will be interesting from the point of view of human health, and how will our ability routinely to monitor their expression and function lead to beneficial changes in medical care?
- Will the ability to quickly monitor the expression of human genes lead to a new era of drug discovery?
- How will we deal with our rapidly increasing ability to detect genetic diseases and polymorphisms and associate mutations with diseases not conventionally thought to be genetic (such as the susceptibility to HIV or malaria) versus the much slower progress in developing effective treatments or preventive measures?
- What will the reaction of the insurance industry be to the ability to easily and cheaply monitor an individual's susceptibility to disease? How should the clinical research community respond to this?

The future of DNA chip technology

The DNA chip promises to be a powerful approach for parallel analyses of hundreds of thousands of distinct sites of the genome, or even for sequencing a gene to detect previously unknown mutations

(Box 2) or to select the appropriate therapeutic approach for a genetic or infectious disease. For better or worse, DNA chip technology will probably bring to reality the promise of genetic screening, prediction and diagnosis that has in many cases been thwarted by the large number and the complex nature of the mutations responsible for genetic diseases. If the chip is used as a tool for molecular research (particularly for large epidemiological studies), it will probably allow associations to be made with mutations and diseases at a population level not possible with existing technology.

Like most new technologies, however, the advances will probably be accompanied by some thorny ethical questions. The biggest 'downside' to the new diagnostics might be the easy availability of test results without approprate counseling to fully understand the results. Gary Cutting remarks that it is not clear to him whether we have the resources in genetics 'to counsel adequately everyone who is likely to want testing, particularily if genetic testing becomes more widespread with the new technologies.' Another ethical issue relates to the ability to detect a genetic condition for which there is no treatment, such as Huntington's disease or Alzheimer's disease. Cutting notes that an individual might prefer not to know that these diseases are going to be a problem, and warns 'we should proceed cautiously.'

The future certainly looks rosy for the companies developing the new chip technologies. 'It's really a new diagnostic market,' says Thane Kreiner of Affymetrix. 'Our sense is that it is the wave of the future, because it will enable physicians to not prescribe drugs that are not working or make bad or very expensive therapeutic decisions.' From a business perspective, its market share 'could eventually be as large or larger than the entire current diagnostic market.' Kreiner thinks that initally the new chip technologies will be used in addition to much of the standard diagnostic testing, but 'in the long term it may be sufficient on its own.' With regard to potential ethical issues, Kreiner is concerned and points to careful placement of the technology: 'I think that making a choice about a drug that works versus one that doesn't, in the case of HIV for example, is a good thing.'

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RELATED PROCEEDINGS APPENDIX

There are no other appeals or interferences related to the instant appeal.